ART 34 AMDT

International Patent Application No. PCT/DK99/00562
Our ref: 22129PC1, Improved method for redistribution
BioImage A/S

CLAIMS

1. A method for extracting quantitative information relating to an influence on redistribution of at least one component in the cell in mechanically intact or permeabilised living cells, the method comprising

recording variation in spatially distributed light emitted from a luminophore, the luminophore being present in the cells and being capable of being redistributed in a manner which is related with the degree of the influence, and/or of being modulated by a component which is capable of being redistributed in a manner which is related to the degree of the influence,

as a change in light intensity wherein the illumination is provided by a laser which is scanned in a raster fashion over some or all of the spatial limitations being measured, the scanning taking place at a rate substantially faster than the measurement process such that the illumination appears to the measurement process to be continuous in time and spatially uniform over the region being measured.

2. A method according to claim 1, wherein the quantitative information which is indicative of the degree of the cellular response to the influence or the result of the influence on the sub-cellular component is extracted from the recorded variation according to a predetermined calibration based on responses or results, recorded in the same manner, to known degrees of a relevant specific influence.

3. A method according to claims 1 or 2, wherein the influence comprises contact between the mechanically intact or permeabilised living cells and a chemical substance and/or incubation of the mechanically intact or permeabilised living cells with a chemical substance.

4. A method according to any of claims 1-3, wherein the cells comprise a group of cells contained within a spatial limitation.

5. A method according to any of claims 1-4, wherein the cells comprise multiple groups of cells contained within multiple spatial limitations.

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6. A method according to any of claims 1-5, wherein the spatial limitations are spatial limitations arranged in one or more arrays on a common carrier.

7. A method according to claim 6, wherein the spatial limitations are wells in a plate of microtiter type.

8. A method according to any of claims 1-7, wherein the redistribution results in quenching of fluorescence, the quenching being measured as a decrease in the intensity of the fluorescence.

9. A method according to any of claims 1-8, wherein the redistribution results in energy transfer, the energy transfer being measured as a change in the intensity of the luminescence.

10. A method according to any of claims 1-8, wherein the intensity of the light being recorded is a function of the fluorescence lifetime, polarisation, wavelength shift, or other property which is modulated as a result of the underlying cellular response.

11. A method according to any of claims 1-10, wherein the light to be measured passes through a filter which selects the desired component of the light to be measured and rejects other components.

12. A method according to any of claims 1-11, wherein the flourescence comes from a fluorophore encoded by and expressed from a nucleotide sequence harboured in the cells.

13. A method according to any of the preceding claims, wherein the flourescence comes from a luminescent polypeptide, such as GFP.

14. A method according to any of the preceding claims, wherein the luminescent polypeptide could be a GFP selected from the group consisting of green fluorescent proteins having the F64L such as F64L-GFP, F64L-Y66H-GFP, F64L-S65T-GFP, and EGFP.

15. A method according to any of claims 1-14, wherein the cells are selected from the group consisting of fungal cells, such as yeast cells; invertebrate cells including insect cells; and vertebrate cells, such as mammalian cells.

16. A method according to claim 15, wherein the mechanically intact or permeabilised living cells are mammalian cells which, during the time period over which the influence is observed, are incubated at a temperature of 30°C or above, preferably at a temperature of

from 32°C to 39°C, more preferably at a temperature of from 35°C to 38°C, and most preferably at a temperature of about 37°C.

17. A method according to any of claims 1-16, used as a screening program.

18. A method according claim 17, wherein the method is a screening program for the identification of a biologically active substance that directly or indirectly affects an intracellular signalling pathway and is potentially useful as a medicament, wherein the result of the individual measurement of each substance being screened which indicates its potential biological activity is based on measurement of the redistribution of spatially resolved luminescence in living cells and which undergoes a change in distribution upon activation of an intracellular signalling pathway.

19. A method according to claim 17, wherein the method is a screening program for the identification of a biologically toxic substance as defined herein that exerts its toxic effect by interfering with an intracellular signalling pathway, wherein the result of the individual measurement of each substance being screened which indicates its potential biologically toxic activity is based on measurement of the redistribution of said fluorescent probe in living cells and which undergoes a change in distribution upon activation of an intracellular signalling pathway.

20. A set of data relating to an influence on a cellular response in mechanically intact or permeabilised living cells, obtained by a method according to any of claims 1-19.